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EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF THE ORGANS IN THE EMBRYO OF THE FOWL (GALLUS DOMESTICUS).

FRANK R. LILLIE.

I. INTRODUCTION.

The results to be described under the above title relate to the morphology, functions and power of regeneration of various embryonic organs, and to the influence that certain embryonic parts exert on the development of others. They represent the application of a particular experimental method, viz., the destruction of definite parts, and study of the subsequent development. Thus the particular organs studied are those most accessible to operation, which form a rather heterogeneous assemblage. Nevertheless, taken as a whole, the results form a contribution to the subject of *correlative differentiation* of organs.

The Principle of Correlative Differentiation in Embryology (i. e., influence of the intraorganic environment in development)¹ is that the rate, degree or mode of differentiation of any embryonic rudiment is dependent on some part or parts of the same organism (individual) external to itself; that is, that component parts of an embryo determine mutually to a greater or lesser extent, their respective lines and grades of differentiation. Much more is meant by this than that any embryonic part can develop only in its normal environment, which offers the prerequisites of its very existence. The principle of correlative differentiation in fact implies a distinction between *a determinative and a non-determinative environment*, and the problem of correlative differentiation is so far resolved when this is ascertained for all the organs (cf. Roux).

Any part, the entire environment of which is non-determinative, is said to develop by *self-differentiation* (Roux).

These two principles do not stand in the relation of rival theories but rather, probably, of coöperative factors in every

¹ Environment may be defined as conditions that influence dynamic processes in protoplasm, and may be divided into extraorganic and intraorganic, the former being external to the individual and the latter within its bounding surfaces.

embryonal differentiation, for any process of self-differentiation of a structure might be analyzable into correlative differentiation of its parts.

For the development of the higher animals at least the extra-organic environment is non-determinative. The development of the ovum as a whole is therefore a process of self-differentiation. But it is usually assumed that it is otherwise with the differentiation of its constituent parts; the extreme view being that each influences the mode of differentiation of all the remainder. From this standpoint the complexity of the correlative processes of differentiation must increase in proportion to the increase in complexity of structure.

Theoretically, at least, the determinative value of correlative differentiation in any case may be (1) absolute, *i. e.*, the mode of development of a part being determined entirely from without itself; (2) partial; (3) wanting, *i. e.*, absolute self-differentiation.

Our present knowledge is enough to exclude the first theoretical possibility. No principle in embryology is better established than that sooner or later the embryo is a mosaic of embryonic rudiments, each of which is to a certain extent self-determining. This mosaic of rudiments may become visible very early, as in those ova exhibiting a definite cell-lineage of organs, or it may appear later. In some cases, at least, the unsegmented ovum itself is a simple mosaic (ovum of ctenophores according to Fischel; ovum of *Unio*, Lillie; ovum of sea-urchins, Boveri; ovum of frog, Roux, Schulze and others). Indeed it is quite probable that all ova are more or less simple mosaics of embryonic rudiments.

Unless, therefore, we wish to beg the entire question we must proceed on the second hypothesis. This is the writer's standpoint, and the problem is to determine as many definite correlations as possible and to investigate their nature.

There is probably no conception in embryology so vague as that of correlative differentiation, as the following citations may serve to show :

Hertwig : "Zelle und Gewebe," II. :

"Die Wechselwirkungen (Correlationen) zwischen den Zellen eines Organismus und ihren Derivaten bilden sich mit dem

Beginn des Entwicklungsprocesses aus, ändern sich von Stufe zu Stufe und compliciren sich in demselben Maasse, als die Entwicklung fortschreitet.

“ Im Gegensatz zum Mosaiktheorie von Roux und der keim-plasma theorie von Weismann stellt die Theorie der Biogenesis den Grundsatz auf, dass vom ersten Beginn der Entwicklung an die durch Theilung des Eies sich bildenden Zellen beständig in engster Beziehung zu einander stehen, und dass dadurch die Gestaltung des Entwicklungsprocess sehr wesentlich mit bestimmt wird. *Die Zellen determiniren sich zu ihrer späteren Eigenart nicht selbst, sondern werden nach Gesetzen die sich aus dem Zusammenwirkung aller Zellen auf den jeweiligen Entwicklungsstufen des Gesamtorganismus ergeben, determinirt.*”

Herbst: “ Formative Reize in der Tierischen Ontogenese: ”

“ Die Aufgabe des zweiten Theiles meiner Abhandlung über die formativen Reize war es also, in der tierischen Ontogenese, abgesehen von der Namhaftmachung jener wenigen Fälle von Ge- kommen von formativen Reizwirkungen, die von irgend einem Teil des Organismus auf einen oder mehrere andere ausgeübt werden, festzustellen und eventuell die Möglichkeit der vollständigen Auflösung der ganzen Ontogenese in einer Reihe von solchen Induktionserscheinungen nachzuweisen.

“ So ist es zum Beispiel zum mindesten ungenau, von der ‘ weitgehenden Wechselbeziehung ’ zu sprechen, ‘ die zwischen allen Theilen eines Organismus auf allen Stadien seiner Entwicklung besteht ’ (Hertwig: ‘ Evolution und Epigenesis ’); denn das Ektoderm der Echiniden entwickelt sich unabhängig vom Entoderm, und auch abgeschnürte Hautstücke, etc., können sich selbständig differenzieren, wie dies das Vorkommen der Teratome beweist (Roux). Die Annahme einer ganz allgemeinen Korrelation zwischen allen Theilen des Organismus auf allen Stadien der Ontogenese ist deshalb ebenso falsch wie jene von der qualitativ ungleichen Kernteilung der Mosaiktheorie.”

Most of the real illustrations (*i. e.*, experimentally determined) of this principle must be taken from plants and plant-like animal colonies. One need only glance through Herbst’s recent “ Formative Reize in der Tierischen Ontogenese ” to realize that, so far as egg development is concerned, the application of the prin-

ciple rests very largely on inference, analogy and a few doubtful pathological conditions.

Discussion of this subject belongs, however, to the conclusion rather than to the introduction, and the foregoing remarks are intended only to define the problem.

II. METHODS OF OPERATION.

In making the operations one must work as far as possible under antiseptic conditions. Instruments, etc., must be sterilized; this is most readily done by passing the needles, knives, scissors, etc., through a flame immediately before each is used. In spite of all precaution a great many eggs are infected. In my experiments only about 20 per cent. of the eggs remained alive until the time of examination for the results of the experiment, two to five days after the operation. The causes of the mortality in the remaining 80 per cent. are two: (1) Fatal injury of the operation (about 40 per cent. ?); (2) infection with mould or bacteria (about 40 per cent. ?). There is a very noticeable difference between different lots of eggs; some bear operations much more readily than others and are less prone to infection. These differences in the relative powers of resistance of different lots of eggs are due to the relative freshness of the eggs when incubation is begun, and also to the time of year. It is noticeable that in a lot of eggs in which a relatively large proportion, over 50 per cent., fail to develop in the incubator, the percentage of failures in the actual experiments is usually very high.

The method of procedure in my experiments was as follows:

1. The eggs are not turned in the incubator, so that one may be sure of locating the position of the embryo in the unopened egg exactly. The upper side of each egg is marked with a pencil.
2. A small opening is made through the shell and membrane over the embryo.
3. The operation is then made. For cauterization I employ either a needle heated red hot in the flame, or an electric cauterizing needle. The heated needle cools very rapidly, so that the operation must be hastily performed, and it is difficult precisely to delimit the injury. The electric cautery, on the other hand, is apt to give too intense heat. Each method possesses certain advantages.

4. The opening in the egg is closed as follows : A piece of the shell with membrane attached is cut from a corresponding part of another fresh egg, so as to be slightly larger than the opening in the operated egg. This is placed over the opening so as to close it completely ; and the albumen adhering to the membrane acts as cement. To ensure perfect closure strips of the egg-membrane are plastered on so as to overlap all edges of the foreign shell. The advantages of this method of closure are that the foreign surfaces are perfectly aseptic if fresh eggs are used, and that the conditions are as nearly like the normal as possible. It is, moreover, the simplest and easiest method. This method of closing the opening was first used by Miss Peebles.¹

III. EXPERIMENTS ON THE AMNION AND THE PRODUCTION OF ANAMNIOTE EMBRYOS IN THE CHICK.

A. *The Normal Development of the Amnion.*

The purpose of this section is to give a brief statement of some facts concerning the formation of the amnion before taking up the analysis of the processes by experiment. This is necessary because the facts are at least partly new, and without knowledge of them the mechanics of formation of the amnion cannot be understood. For a recent review of the literature on the whole subject of the amnion in the Sauropsida, see Schauinsland ('02a and '02b) ; the latter paper I regret not to have seen.

In the somatopleure on each side of the axis of an early embryo of the chick three zones may be distinguished on the basis of the subsequent differentiation, (*A*) for the body-wall ; (*B*) for the amnion ; (*C*) for the chorion (serosa) (Fig. 1). It is important to trace the origin of the differentiation between the amnion and serosa on the one hand, and amnion and body-wall on the other, for the conditions that determine the development of the amnion must be antecedent to such differentiation.

1. *The Ectamnion.*—The differentiation of the amniogenous from the choriogenous somatopleure is always preceded by the appearance of a thickening of the ectoderm along the external margin of the former. This thickening, for which I propose the

¹ Roux's *Archiv*, VII., 1898.

name ectamnion, precedes by a little the formation of amniogenous folds in any region, and indeed it induces the origin of the entire system of folds. It has been described by many embryologists at the stages immediately preceding fusion of the limbs of

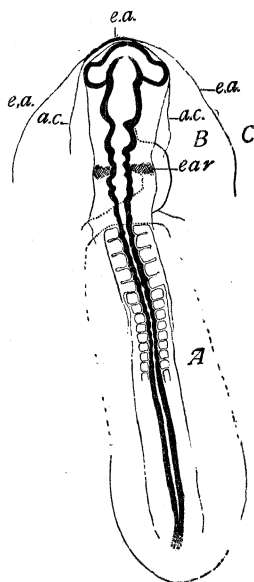


FIG. 1. Embryo of chick with 13 mesoblastic somites. University of Chicago Embryological Collection, No. 555. *e.a.*, ectamnion; *a.c.*, inner margin of amniocardiac vesicles; *A*, region of the somatopleure destined to form the body-wall; *B*, amniogenous somatopleure; *C*, choriogenous somatopleure.

the amnion (cf. Schenk, '71), and it forms the ectodermal sero-amniotic connection of Hirota ('94). But no one, so far as I know, has traced it back to its origin and recognized the fact that it is the earliest formed part of the amnion, which is thus primarily ectodermal in the chick, as in *Chelonia* and some other primitive Sauropsida.

The ectamnion may first be distinguished at about the stage with nine mesoblastic somities, where it appears as a median thickening of the ectoderm in front of the head near the anterior boundary of the proamnion. Along the line of this thickening there is a fusion, between ectoderm and entoderm. The thickening is extended right and left and turns backwards along opposite sides of the head to about the region of the middle of

the heart, gradually becoming more peripheral in position and slowly fading out (Fig. 1). This line represents the junction of the amniogenous and choriogenous somatopleure, and thus corresponds to the angles of the future amniotic folds.

The head of the embryo lies in a depression bounded in front by the ectamnion and on the sides by the amnio-cardiac vesicles of the body cavity, along the inner upper margin of which the ectamnion runs for a short distance. The floor of the depression is the proamnion.

In a stage with 14-15 mesoblastic somites the ectoderm of the proamnion is much more thickened in front of the head, and has

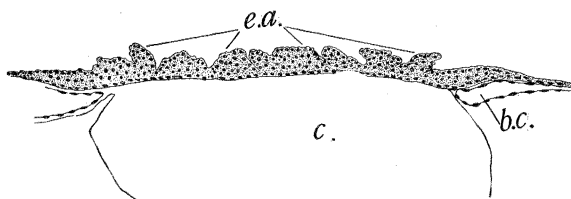


FIG. 2. Transverse section through the anterior angle of the ectamnion, a few sections in front of the tip of the head. 14-15 mesoblastic somites. University of Chicago Embryological Collection, No. 215. *b.c.*, body-cavity; *c.*, large cavity in the entoderm; *e.a.*, ectamnion.

a *villous outer surface* in consequence of irregularity in the thickening¹ (Fig. 2), which may be traced back to the level of the heart, and on one side to its hinder end; there is also a very short ectentodermal fusion beneath the tip of the head. In this series the ectamnion marks the boundary between two distinctly differentiated parts of the extraembryonic somatopleure, the more central of which is the amnion.

In another embryo with fourteen mesoblastic somites, the tip of the head is surrounded by the amnion, and the proamniotic part is represented only by a short median strip extending eight sections back to a point where the limbs of the amnion have not yet closed. The ectamnion is continued only for a short distance along the *angles* of the amniotic fold, and then passes peripher-

¹ In examining the section one receives a strong impression that the irregularities may be due to amoeboid movements; but it is not possible to confirm this by actual observations.

ally. How has the head-fold been formed? The great expansion of the body cavity (amniocardiac vesicles) on each side causes an elevation of the anterior angle of the ectamnion and a pocket is formed by fusion of its opposite limbs, which have a strong affinity for each other; fusion proceeds along the median dorsal line so long as the energy of fusion is sufficient to draw the somatopleure up. The head of the embryo is rapidly elongating at this time and slips into the pocket thus formed, being guided in part by the cranial flexure (Fig. 1). It is interesting to note how far the ectodermal thickening stretches ahead of the mesoderm of the fold near the point of closure, and that the apical cells are elongated into pseudopodium-like processes.

The histological differentiation of the amniotic area of the somatopleure from the chorionic portion precedes the elevation of the fold.

This brief inquiry, then, suggests that the order of events in the formation of the head fold of the amnion is:

1. Thickening of the ectoderm on the outer margin of the amniogenous somatopleure, beginning in front of the head of the embryo and extending back on each side (ectamnion).
2. Great expansion of the body cavity on each side opposite the head of the embryo and consequent elevation of the anterior bay of the ectamnion to the level of the dorsal surface of the embryo.
3. Fusion of the right and left limbs of the ectamnion, beginning at the angle, to form a pocket, the head-fold of the amnion.
4. Pushing of the head of the embryo into the fold.

There may be, however, considerable variation in the time of formation of the head-fold. I have, for instance, one series with 17-18 mesoblastic somites (ser. 175), where the head-fold is not yet formed.

Extension of the Ectamnion.—The ectamnion differentiates backward more rapidly than the lateral folds, and always precedes their origin. In the 48-hour stage (21-22 somites) (Fig. 3) the ectamnion from in front has joined that from behind formed in connection with the tail-fold. There is a place, corresponding nearly to the final meeting place of anterior and posterior lateral folds, where it becomes very faint. It would appear

then that behind the tail there is actually a new starting-point for the ectamnion as well as the amniotic folds. The primary position of the ectamnion is near the boundary of the pellucid area; towards the posterior end it bends in very sharply, nearly joining the body wall proper, and terminating in the posterior rudiment.

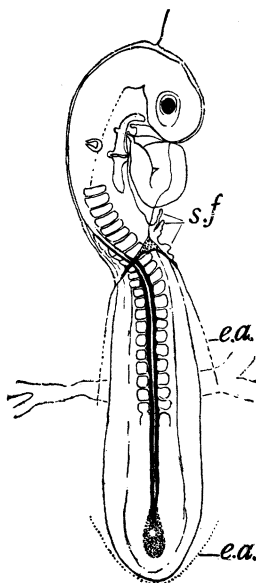


FIG. 3. Embryo of chick with 21 mesoblastic somites. University of Chicago Embryological Collection, No. 99. *e.a.*, ectamnion; *s.f.*, secondary folds of the amnion on the right side. The dotted line continuing *e.a.* represents the continuation of the ectamnion beyond the region of folding. The dotted area at the angle of the folds represents the ectodermal sero-amniotic connection of Hirota.

Origin of the Tail-Fold. — The tail-fold proper arises from an ectodermal thickening lying in a depression just beneath the rudimentary tail-bud. The depression is caused by the enlargement of the body cavity on each side of the middle line. These enlargements may be called the amnio-allantoic enlargements, as they are associated with the formation of the allantois. I would venture the hypothesis that the existence of a *separate* tail-fold of the amnion is associated with the time of development of the allantois, which is represented in the embryo under consideration (1) by a shallow entodermal evagination and (2) a mass of mesoblast.

At the time of formation of the tail-bud a very shallow pocket forms behind it. This owes its origin to the elevation of lateral folds of the somatopleure and progressive fusion beginning at the posterior angle of the ectamnion. The floor of the pocket includes a thick posterior prolongation of the allantoic mesoblast which furnishes a firm floor to the pocket and thus determines the form of the folds.

2. *The Amniotic Folds.*—The subsequent development includes the elevation and fusion of the anterior and posterior lateral folds. The final closure takes place opposite the buds of the hind limbs. The order of events in these processes is as follows :

1. The growth of the amniogenous somatopleure behind the head-fold and in front of the tail-fold.
2. The uprising of the amniotic folds, and their growth in a definite direction around the embryo.
3. The fusion of the right and left folds along the line of the ectamnion in such a way that the external limbs unite to form the chorion, and the internal to form the amnion.

Study of the morphology of these processes suggests the following physiological conclusions :

1. The growth of the amniogenous somatopleure may be a result of the traction exerted in it by the progressive fusion of the folds already formed in front and behind.
2. The uprising of the lateral folds is determined by the head- and tail-folds, the progressive fusion of the right and left ectamnion dragging the amniogenous somatopleure into place.

It remains to test these conclusions by experiments, but before proceeding to a description of these, I wish to describe *the influence of the rotation of the embryo on the amniogenous somatopleure.*

Practically all of the somatopleure of the pellucid area is amniogenous with the exception, naturally, of that part internal to the limiting sulci that forms the body-wall. What effect has the turning of the embryo on its left side on the amniogenous somatopleure? We will suppose that the latter is primitively of equal width on both sides ; we will furthermore assume that the somatopleure cannot be drawn in from the vascular area, because it is here attached to the splanchnopleure. (The fusion of the somatopleure and splanchnopleure at the margin of the pellucid

area is shown by the fact that the splanchnopleure is often drawn up with the outer limb of the amniotic fold, making a fold of the splanchnopleure at this place) (Fig. 5). Finally let us assume that the notochord represents approximately the axis of rotation. During the process of rotation the embryo sinks and the lateral limiting sulci become deeper. A direct consequence of the rotation must be therefore a strong tension on the somatopleure belonging to the under (left) side, *a-b*, and practically none on the upper (right) side, *c-d*, (see Fig. 4, *A*, *B*, *C*).

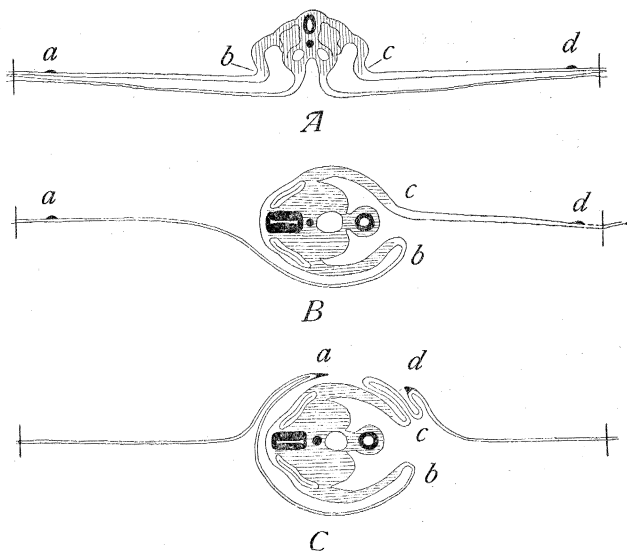


FIG. 4. *A*, *B* and *C*. Diagrams to represent the effect of rotation of the embryo on the amniogenous somatopleure. *a* represents in all figures the position of the ectamnion on the left (lower) side; *d* represents in all figures the position of the ectamnion on the right (upper) side. *b* and *c* represent the junction of amnion and body-wall on left and right sides respectively. In Fig. *A*, *a-b* and *c-d* are equal. In Fig. *B*, rotation of the embryo is assumed to have taken place without formation of the amnion; the distance *a-b* has become greater than *c-d*. In Fig. *C* is represented rotation of the embryo with synchronous formation of the amniotic folds, as is actually the case; *c-d* is inevitably thrown into secondary folds. The vertical lines at the extreme right and left represent the margins of the pellucid area.

Even though the difference may be partly compensated for by drawing of the embryo to the left, the tendency would be to stretch *a-b*. If there were no such compensation and *a* and *b* were practically fixed points, the length of *a-b* at the conclusion

of the rotation would much exceed that of $c-d$ (Fig. 4, b); and if during this process there were actual independent growth of $a-b$ and $c-d$, the latter would of necessity be thrown into folds, but not the former. Finally, if the amniotic folds were forming at the same time (as is actually the case) the right one would inevitably be thrown into secondary folds by the approximation of points c and d (Fig. 4, C).

Study of the fusion of the amniotic folds in actual section shows (1) that the line of fusion of the opposite amniotic limbs is over the dorsal surface of the embryo *only so long as the latter lies flat on the yolk*, and does not follow the turning of the embryo on to (usually) its left side; the consequence is that after rotation of the embryo the line of fusion lies over the upper (right) side of the embryo, often opposite the horizontal level of the intestine

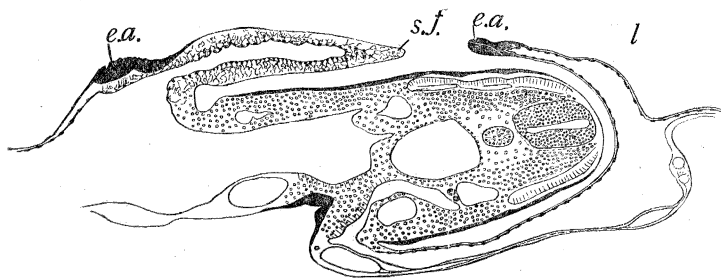


FIG. 5. Transverse section of an embryo of about 48 hours (Duval) showing the position of the ectamnion on the right and left sides. University of Chicago Embryological Collection, No. 689. *e.a.*, ectamnion; *l.*, left; *s.f.*, secondary fold of amnion on the right side. The great differences in the thickness of the amnion of the right and left sides should be noted.

(Fig. 6). Thus one fold of the amnion passes all the way from the under side over the back of the embryo and around on the other side to the line of fusion, and thus is several times as long as the opposite limb. (2) Moreover, the amniotic fold of the right side is invariably thicker than that of the left side, and is always thrown into secondary folds at the place of turning (Fig. 5 and Fig. 6). These conditions are satisfactorily explained, as noted above, by the mere turning of the embryo on its side.

One must therefore distinguish in the upper limb of the amnion two kinds of folds: (1) The ordinary amniotic fold induced by the fusion of the right and left rudiments and (2) *secondary*

folds formed simply by the process of twisting of the embryo. This distinction is of importance in interpreting the results of the experiments.

Hirota (94) notices the secondary fold on the upper side and says: "It seems to owe its origin to the presence of the sero-amniotic connection. . . . It is always on the right side of the connection, and is pushed on towards the left. There takes place no folding before the allantois appears, and the longitudinal ex-

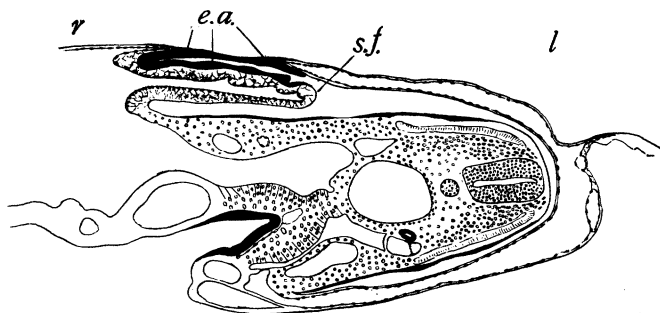


FIG. 6. Section of the same embryo as the preceding, 10 sections (150μ) in front of Fig. 5. The section passes through the place of fusion of the right and left folds. The secondary fold of the amnion is well shown on the right side. Letters as in Fig. 5.

tent of the fold depends on the extent of the sero-amniotic connection." "Its form and extent are variable." "It is not clear what significance this fold has." "At both extremities of the sero-amniotic connection the amnion is also slightly folded longitudinally."

These secondary folds of the amnion are very transitory except in two regions: (1) Above the hind end of the heart (apex of ventricle) and continuing a short distance behind it; (2) in the region immediately in front of the allantois, at 60-70 hours, thus in the neighborhood of the final closure of the amniotic folds. The former are of very constant occurrence and persist a long time (Fig. 3). The latter are relatively slight and inconstant. Hirota is thus mistaken in saying that these folds do not appear until the formation of the allantois.

The secondary folds in the neighborhood of the heart are always on the upper (right) side; they first appear at the time

of rotation of the embryo, and are coincident with the closure of the amnion (Fig. 3); they persist until the body-wall is completed behind the entire heart. They are not, in my opinion, exclusively folds of the amnion, but extensions of the body-wall for enclosure of the region of the heart and liver. The direct cause of their formation is, however, the rotation of the embryo with extreme growth of the body-wall contiguous to the amnion, and fixation of the outer end of this limb of the amnion by the amniotic suture.

Elsewhere the effect of the twisting of the embryo is rapidly compensated so that the secondary folds of the right half of the amnion do not persist long except in the region of the allantois, where slight inconstant secondary folds may continue longer.

B. *Experimental.*

1. *Experiments on the Head-fold of the Amnion.*

Experiment No. 57.

Age of the embryo at the time of operation, 33 hours¹ (Duval).

Operation.—The blastoderm was cauterized lateral to the right optic vesicle with a needle (Fig. 7) so as to make a large opening. At the time of the operation only the most anterior horse-shoe-shaped segment of the ectamnion was present (cf. Fig. 1), and this was destroyed only on the right side of the embryo. On the left side, therefore, the amniotic fold was free to form to the extent that it is independent of the opposite fold. The right optic vesicle was slightly injured, as the results of the experiments show. In opening the egg for the operation, the blastoderm was

¹ In describing the various experiments, the age of the embryo at the time of the operation will not be given as the actual number of hours in the incubator, because the variations in point of actual development after the same period of incubation are so extreme. It is not possible either to make accurate measurements of the living embryo or to determine the number of somites present, on account of the loss of time and danger of exposure of the embryo. A rough sketch of the embryo was always made at the time of the operation, and this is sufficient to identify it with the various stages figured in Duval's atlas. The age is based on this identification. Thus the given age at the time of operation in these experiments represents a certain definite stage of development. On the other hand, the length of time that elapsed from the experiment to the time of reopening the egg is always given literally.

also inadvertently torn just back of the embryo, and this opening also appears in Figs. 8 and 9. This, however, was without any noticeable effect on the subsequent development.

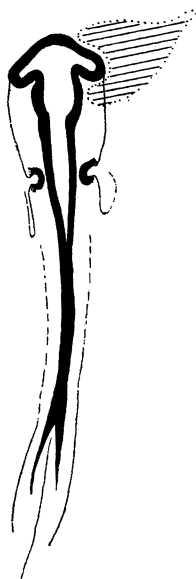


FIG. 7. Experiment 57. Operation diagram. Outline of embryo of chick or about 33 hours, after Duval. The ruled area to the right of the head indicates the area of the blastoderm destroyed by the heated needle.

Examination of the Resulting Embryo.—The egg was reopened 48 hours after the operation. The heart was beating vigorously; the hole made in the blastoderm by the operation had not closed, and a good deal of yolk had escaped through this and overlay the blastoderm. The embryo was well developed, corresponding to the stage of 70–80 hours (Duval), and apparently normal in all essential respects. (A defect in the right eye was evidently a direct result of the operation.) The head of the embryo had slipped through the hole in the blastoderm and was suspended in the yolk (Figs. 8 and 9).

The embryo was cut into 250 sections of 15μ thickness. Around the edges of the opening made by the operation the somatopleure turns over and becomes continuous with the splanchnopleure, ectoderm with entoderm, and mesoderm with

mesoderm. In places one cannot determine where the ectoderm leaves off and the entoderm begins.

Amniotic Rudiments of the Left Side.—A short distance in front of the margin of the opening there is a sharply defined fold of the somatopleure capped by an ectodermal thickening that

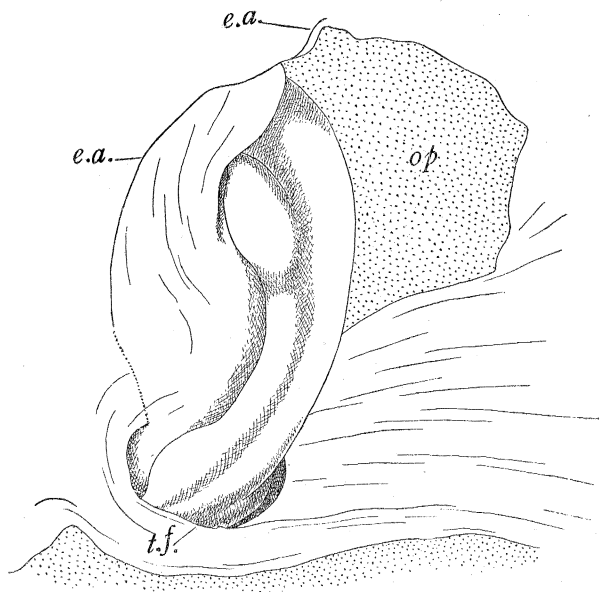


FIG. 8. Experiment 57. Upper surface of blastoderm. *op.*, aperture in the blastoderm made by the operation; *e.a.*, amniotic rudiment of the left side; *t.f.*, tail-fold of the amnion. The stippled area behind the embryo represents an aperture in the blastoderm accidentally made in opening the egg for the operation.

represents the head-fold and left lateral fold of the amnion. The extent of this fold is indicated by the line *e.a.* on Fig. 8. It begins as a sharply marked fold at the most anterior angle of the opening, and passes back, at first along the edge of the opening, later a short distance from it, to the left of the embryo. It very distinct (Fig. 10, *l.a.f.*) to the point where it is indicated as is a broken line; in this region the fold has disappeared, but the thickening of the ectoderm (ectamnion), may be traced back to the tail-fold with which it becomes continuous as indicated in the drawing (Fig. 8). At no place, until the tail-fold is reached, is the somatopleure internal to this line thrown into folds. By

reference to the figure and to the description of the operation it will be seen that the line of this fold represents the continuation of the left amniotic rudiment, which was not injured by the operation.

I conclude, therefore, that when the amniotic rudiment of one side is left free to develop after destruction of the rudiment of the other side just prior to the formation of the head fold,

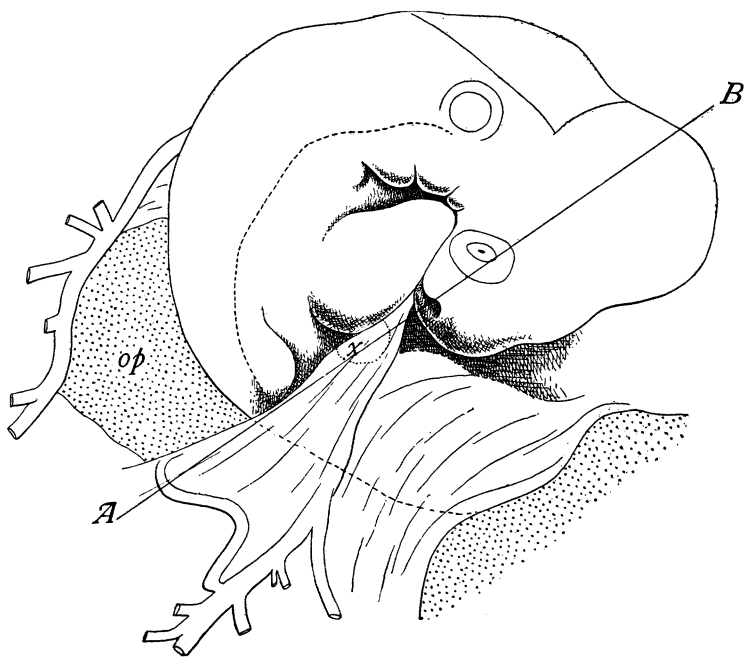


FIG. 9. Experiment 57. Under surface of the blastoderm. There is no amnion. The right eye is defective. *x* marks the location of the secondary amniotic fold shown in Fig. 10. *A-B*, plane of section shown in Fig. 10. Letters as in Fig. 8.

the ectamnion is propagated in the normal fashion and induces the formation of a low fold, but that the amniogenous somatopleure is unable to raise itself around the body of the embryo. The growth of the amniogenous somatopleure appears to be less than normal.

Amniotic Rudiments of the Right Side. — On the right side, on the other hand, a well-developed fold appears at the place where the extra-embryonic somatopleure becomes continuous with the

body-wall (section 138, Fig. 10) and extends to section 167, where it suddenly ceases, a distance of about 0.5 mm. The location is indicated by *x* on Fig. 9, and Fig. 10 shows it in section.

The formation of this fold is not induced by the ectamnion because the line of the latter (Fig. 10, *r.e.a.*) may be recognized some distance lateral to the fold, through it is very slightly developed. The fold in question is immediately back of the heart on the right side of the body. It is not, in my opinion, a true amniotic fold, but belongs to the category of normal secondary folds of the amniogenous somatopleure produced by the turning of the embryo, with which it agrees precisely in position and appearance. This conclusion is reinforced by the following consideration : in this embryo the roots of the vitelline veins are prolonged forward to an abnormal extent, and the right vein is fused to the somatopleure lateral to the fold (Fig. 10). As the

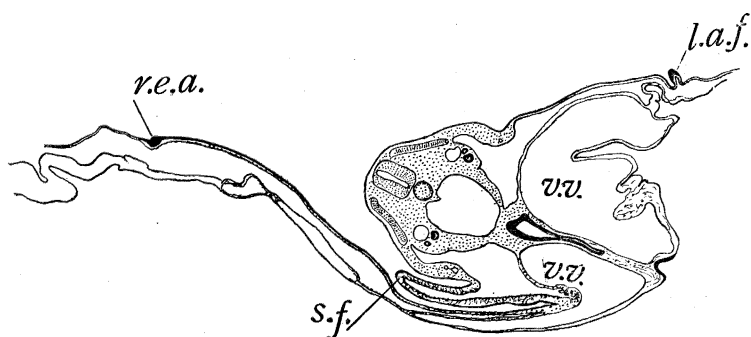


FIG. 10. Section through the embryo of experiment 57 along the line *A-B* of Fig. 9. *l.a.f.*, left amniotic rudiment ; *r.e.a.*, ectamnion of the right side ; *s.f.*, secondary fold of amnion on the right side ; *v.v.*, vitelline veins.

embryo turns, therefore, the somatopleure between the vitelline vein and the body-wall must be folded to the extent that the turning approximates the body-wall to the vein, because the fusion prevents the somatopleure from being pushed peripherally. As already said, therefore, this is not a true amniotic fold.

The prevention of the formation of the head-fold, by destruction of the rudiment of one side, operates to prevent the normal elevation of the amniotic fold on the opposite side ; and thus it is experimentally demonstrated that the coöperation of right and left folds is necessary for the normal mode and direction of

growth of the amniotic rudiments. The height of the fold on the uninjured side is a measure of the power of independent elevation of a single amniotic fold.

On the other hand the existence of the ectamnion on the right side, though in a rudimentary state, and the differences in finer structure of the somatopleure on the two sides of this line indicate that the distinction between amniogenous and choriogenous somatopleure is attained by the normal development of the somatopleure as a whole, and not simply as a result of their separation after fusion. However, the relatively rudimentary condition of the ectamnion on the injured side shows that the earlier stimulate the growth of the latter formed parts; otherwise we should expect to find the ectamnion equally developed on both sides. The ectamnion of the right side does not exactly join the tail-fold.

Tail-fold. — The tail-fold of the amnion may be well seen in Fig. 8. So far from compensating in any way for the absence of head and lateral folds, it is of even less than its normal extent, a fact indicating (possibly) that normally its growth is stimulated by the traction of the anterior section of the amnion.

Experiment No. 36.

Age of the embryo at the time of operation forty-six hours (Duval).

Operation. — The operation consisted in the insertion of a heated needle just in front of the heart (see Fig. 11). Examination of the sections of the resulting embryo shows that the injury involved the left optic cup slightly, and that the head-fold of the amnion which extends back beyond the heart at this stage, stuck to the needle and was stripped off, carrying with it a certain amount of the adjacent somatopleure. This was not observed at the time of the operation, but the conclusion is rendered positive by the subsequent examination of the embryo.

Examination of the Resulting Embryo.

The egg was reopened and the embryo preserved forty-eight hours after the operation. The embryo (Fig. 12) appeared like a normal embryo of about the ninety-sixth hour. The limb-buds were well started, and the allantois extended out beyond

the embryo, but towards the dorsal surface; the flexures were normal. The striking thing was the apparent entire absence of the amnion; the embryo lay naked on the surface of the blastoderm, to which it was attached, in the same manner as a selachian embryo by a very broad somatic and splanchnic umbilicus.

In the normal embryo of this age the amnion is completely closed, and the body-wall of the embryo has, therefore, lost all connection with the chorion.



FIG. 11. Experiment 36. Operation diagram. Outline of embryo of chick of about 46 hours, after Duval. The ruled area shows the site of the operation with the heated needle. For description of the operation see text.

This embryo was cut into 625 transverse sections. These confirm the general absence of the amnion, and at the same time furnish additional data. Back to about the 354th section (forty sections behind the heart), the somatopleure beneath the embryo is entirely missing; evidently it had been torn away by the operation and had not been replaced. Throughout this region the extra-embryonic somatopleure begins on each side of the embryo with a free edge. A short distance behind the heart, folded portions of the original amnion appear lying in the gap in the somatopleure, and continuous with the midventral line of the body-wall. Beginning with about the 371st section (see Fig. 13) the body wall is open ventrally, and is continuous with the

extra-embryonic somatopleure on one side, while on the other the original gap in the somatopleure is still open (see Fig. 13). In this region, the somatopleure for some distance external to

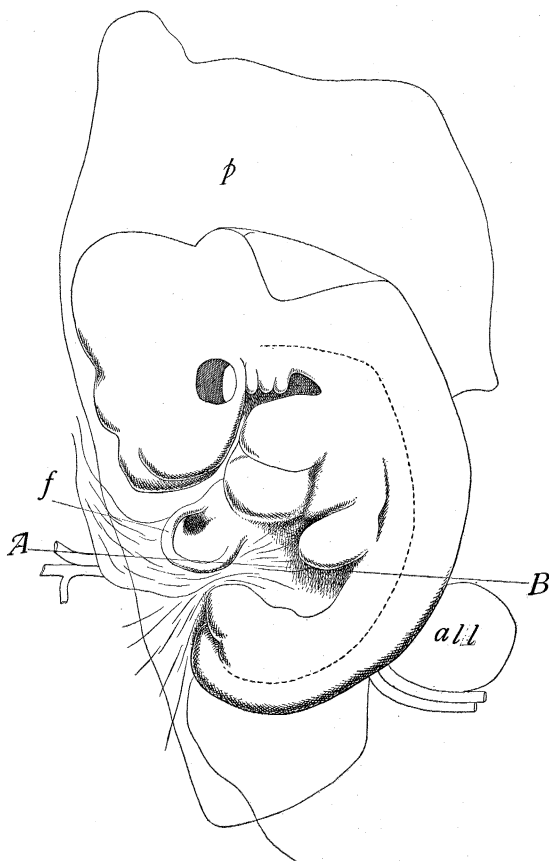


FIG. 12. Experiment 36. Surface view of embryo; upper surface of blastoderm. The embryo is anamniote, except for a rudimentary tail-fold. *all.*, allantois; *p.*, pellucid area. *A-B*, plane of section shown in Fig. 13; *f.*, fold of somatopleure.

the part destined for the body-wall is thrown on both sides into irregular folds that obviously represent the lateral amniotic folds. They rapidly decrease in size posteriorly, and *almost* completely disappear in the region extending from the 420th section back, *i. e.*, a short distance back of the fore-limbs. Beginning opposite the hind-limbs the folds again increase in size. They are very irregular and do not form the normal investment

of the tail. But beneath the latter they form a closed pocket, the usual tail-fold.

Over the entire region, extending from about the posterior edge of the fore-limb to the beginning of the hind-limb, there are no folds in the amniogenous somatopleure. This would indicate that the normal rapid growth of this region is progressively induced under normal conditions by the extension of the lateral angles of the head-fold backwards. The folds shown in the figure are only from about 354-430 and may be explained as remnants of the original head-fold, the postero-lateral prolongations of which were probably not entirely removed by the operation. These folds have not, however, united over the embryo nor have they induced formation of folds behind them. The

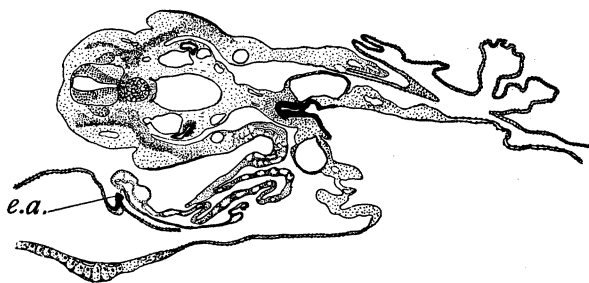


FIG. 13. Experiment 36. Section of embryo along the line *A-B*, Fig. 12. The irregular and incomplete amniotic folds are well shown. *e.a.*, ectamnion of the right side.

reason for this is clear when we consider that the normal process involves continuous traction on the somatopleure back of the advancing folds, for the latter are continually fusing along the dorsal line with those of the opposite side and thus are constantly, so to speak, gathering in the slack, and causing tension.

In the drawing of the entire embryo, the left side is uppermost, but at the time of the operation the right side was up. Evidently the embryo was turned over after removal of the blastoderm in the process of preparation. This explains why in the section the lower amniotic fold has the usual appearance of the upper fold. The ectamnion is visible only on the left side of the drawing; on the right side no trace of it could be found, except in the region of the tail-fold.

The results of the destruction of the head-fold of the amnion in the stage of 46 hours are : (1) Inhibition of the progressive differentiation of the amniotic zone of the somatopleure; (2) failure of the parts of the lateral folds left to unite around the embryo. The failure of the amniotic folds to unite in the region where they are best formed and are of more than sufficient length for enclosure of the embryo shows that the normal union of the folds is due to the guidance and support of the earlier formed parts of the amnion.

The tail-fold, however, forms in a fairly normal manner. The actual abnormalities in this fold are probably secondary, that is, probably due not so much to direct disturbance of the amnion itself as to the freedom of movement of the embryo permitted by the absence of the head-fold, resulting in the withdrawal of the tail of the embryo from the forming tail-fold.

The body-wall is unenclosed for 113 sections; in a normal embryo of about the same age the body-wall is unenclosed for about 55 sections. Thus it would appear that the closure has been delayed.

Experiment 60.

Age of the embryo at the time of operation about 33 hours.

Operation.—The blastoderm was cauterized just lateral to the right optic vesicle, as in experiment 57, producing a large opening (Fig. 14).

The egg was reopened 72 hours after the operation, and a large, finely developed vascular area was seen with apparently no embryo. But more careful examination revealed the naked hind quarters of an embryo sticking up near the center of the vascular area, the whole trunk and head of which were plunged through the blastoderm into the yolk. The head and trunk of the embryo had slipped through the hole made by the operation into the yolk-sac, and the edges of the blastoderm around the original opening had fused in such a way as to close around the hinder part of the embryo. A large part of the vascular area was cut out and the embryo was gently floated into a watch crystal of physiological salt solution. Turning over the blastoderm, the embryo was revealed entirely without an amnion (Fig. 15). Not even the tail-fold was found.

The embryo is represented in Fig. 15 as it lies on the reversed blastoderm, the entodermal face of which is up. The allantois is well developed and lies in a special enlargement of the body cavity behind the embryo.

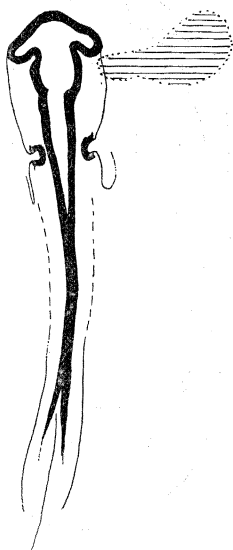


FIG. 14. Experiment 60, operation diagram. Outline of embryo of chick of about 33 hours, after Duval. The ruled area indicates the region of the blastoderm destroyed by the operation.

In this experiment, as in experiment 57, only the right limb of the ectamnion of the prospective head-fold was destroyed; and the consequence of this is in both cases the suppression of the amnion with the exception of the tail-fold. In this case the various membranes have been so confused by the curious position of the embryo and by various secondary fusions that it is quite impossible to determine the behavior of the uninjured rudiment of the amnion of the left side. A single section may serve to illustrate one of the very peculiar conditions (Fig. 16). Lying above the embryo is seen the blastoderm composed of the somatopleure and splanchnopleure. The body-wall of the embryo has fused with the splanchnopleure in such a way that the two are directly continuous on both sides, and the body wall may be traced directly into the wall of the intestine. The result

of this fusion must have been an opening on each side into the yolk-sac; but this has been roofed over by extension of the

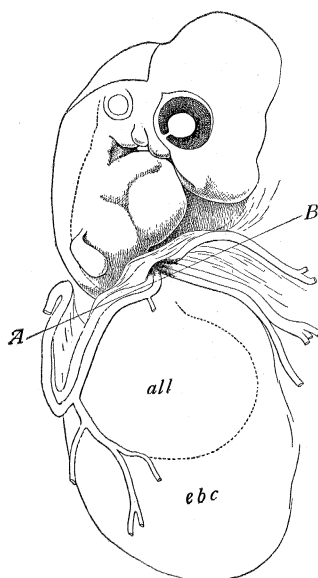


FIG. 15. Experiment 60. Under *i.e.* entodermal, surface of the blastoderm. The embryo is anamniote, but otherwise quite perfect. *all*, allantois; *e.b.c.*, extra-embryonic body-cavity; vitelline arteries and veins shown. *A-B*, plane of the section shown in Fig. 16. The embryo was suspended within the yolk-sac, as described in the text.

blastoderm surrounding it. Farther back the wall of the intestine becomes continuous with the extra-embryonic splanchnopleure.

In the region of the tail rudiments of the tail-fold of the amnion are found.

Two other completely anamniotic embryos (numbers 112 and 124) were produced by experiments similar to those already described. Both of these had passed through the hole made in the blastoderm and were suspended within the yolk-sac. One of these was much farther developed than number 60. They confirm the general results of the dependence of amnion formation on the presence of the head-fold. They possess other definite lesions, the effects of which will be described in another paper.

Condition of the Allantois in Anamniotic Embryos.

The allantois is well formed in four of these embryos; one (No. 57) was too young to show it externally. It is obvious that in the absence of the amnion the growth of the allantois

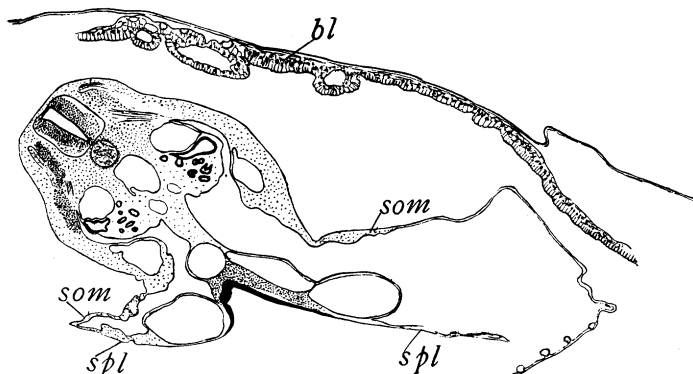


FIG. 16. Experiment 60. Section along the line *A-B*, Fig. 15. *bl.*, blastoderm overlying the embryo; *som*, somatopleure; *spl.*, splanchnopleure. On the right side there is a break in the continuity of somatopleure and splanchnopleure; this was evidently produced in the preparation, as the continuity is perfect some distance in front, and also behind.

must be attended with difficulties. When the amnion is normally formed a large free space is created above and around it, into which the allantois can freely spread. The absence of this space causes compression of the allantois, and changes the direction of its growth, but I do not think that the latter is much impeded. The mechanical force of the expansion of the allantois causes separation of the somatopleure and splanchnopleure to proceed more rapidly in its immediate vicinity than elsewhere (see Fig. 15). In experiment 124 the greater diameter of the allantois exceeds the greatest length of the embryo. I see no reason why this process might not provide all necessary space for its expansion. It might be, however, that the resistance offered would tend to cause accumulation of the products of excretion in the body of the embryo, and thus gradually poison it.

2. *Experiments on the Tail-Fold of the Amnion.*

I have also made a number of experiments on destruction of the tail-fold of the amnion. The results are in most cases com-

plicated by conditions that do not properly belong to the subject of this paper. There is but one uncomplicated case (exp. 18). In this experiment the hind-end of the embryo was cauterized immediately after the appearance of the tail-bud (Fig. 17), thus

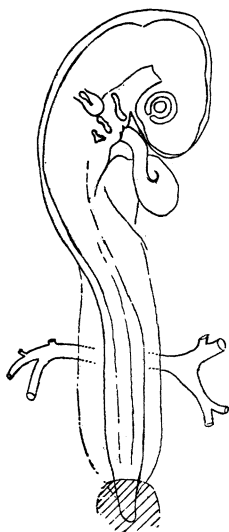


FIG. 17. Experiment 18, operation diagram. Outline of embryo of chick of about 52 hours, after Duval. The ruled area represents the part destroyed by the heated needle.

destroying the tail-fold of the amnion. When the egg was reopened forty-eight hours later, a well-developed embryo of about five days was found in which the amnion ceased with a free edge immediately in front of the hind-limbs (Fig. 18).

The conditions of the membranes in this embryo are otherwise very complicated and difficult to understand. Thus there is in addition to the amnion a fold of the blastoderm surrounding both amnion and embryo (Fig. 18). In the posterior half of the embryo the body-wall is directly continuous with the wall of the intestine as in 60. As this embryo will come up for description elsewhere, I shall not dwell further on this topic.

The fact that stands out distinctly is that the tail-fold of the amnion has not regenerated and that the head-fold has not compensated for the absence of the tail-fold by continuing its growth backwards. However, I have a number of embryos in

which a complete amnion has been found without any tail-fold. These embryos, are, however, defective at the hind end, so that

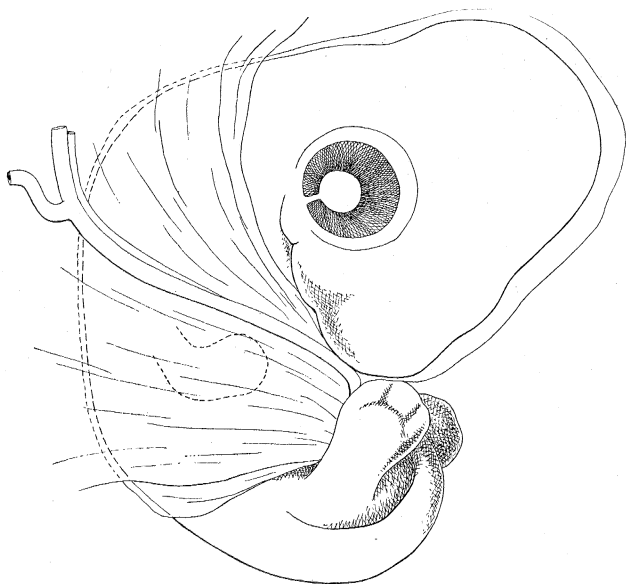


FIG. 18. Experiment 18. The embryo 48 hours after the operation. The tail-fold of the amnion has not regenerated. The amnion ends with a free edge in front of the hind-limbs. A fold of blastoderm is wrapped around the embryo and amnion. Under surface of blastoderm.

one has not to attribute any work of supererogation to the anterior lateral folds to explain the complete closure. This also will be discussed elsewhere.

GENERAL DISCUSSION.

The formation of the amnion of the chick seems to be a process with extraordinarily slight power of regulation.¹ A slight injury to part of its early rudiment sets the whole process astray. It is thus an extremely good example of correlative differentia-

¹ Barfurth ('02) notes incidentally in one of his experiments "die Amnion war regenerirt." As I understand him, he means by this simply that an aperture made in the amnion in the course of an experiment on the eye closed up. I can confirm this from my own observations. I have found that even considerable tears made in the amnion *after its formation* may close completely.

tion. The correlations in the development of the amnion are of three kinds :

1. *Mechanical*. — Under this head I class the elevation of the lateral amniotic folds, which takes place only after the establishment of the head-fold, and which is omitted, if for any reason the head-fold fails to appear or is destroyed.

2. *Trophic Stimulation*. — Under this head I class the influence of the traction exerted by the union of the right and left amniotic folds on the amniogenous somatopleure, and the influence of the turning of the embryo on the amniogenous somatopleure of the left side. The influence of the traction in either case is to increase the extent of the amniogenous somatopleure, in part (presumably) by stimulating its growth, in part undoubtedly by mere stretching. If, owing to failure of formation of the head-fold, such traction is not exerted on the somatopleure it does not expand nearly to the normal extent.

3. *Differential Stimulation*. — Under this head I class (doubtfully) the propagation of the ectamniotic thickening along the somatopleure ; though this may be a process of self-differentiation.

Self-differentiation of the Formation of the Amnion. — The formation of the original rudiments of the ectamnion may be a process of self-differentiation, though the definite relation of the anterior and posterior rudiments to the head and tail respectively suggests correlation with their formation. Moreover, a slight histological differentiation appears between the amniogenous and choriogenous somatopleure, before, and even in the absence of, the formation of folds, which is apparently not correlated with any other of the processes observed.

Beyond this mere classification I do not desire to go at present, but will reserve a general discussion of principles until the completion of other parts of the present series.

In conclusion I simply summarize the results :

I. *Morphological*.

1. The amnion is primarily an organ of the ectoderm in the chick. The ectamnion first forms in front of the head and differentiates progressively backwards towards the posterior end,

where it is met by the posterior ectamnion differentiating forwards. Thus the amniotic zone of the somatopleure is marked off from the chorionic zone.

2. The head-fold is formed from the ectamnion with the coöperation of the amnio-cardiac vesicles and of the proamnion which is depressed between the former. The immediate prolongation of the head-fold is produced by the progressive fusion of the ectamnionic rudiments backwards, and it includes only an extremely small part of the proamnion.

3. The tail-fold is likewise formed primarily by the ectamnion with participation of the amnio-allantoic enlargements of the body-cavity.

4. There are certain constant secondary folds in the upper (right) limb of the amnion produced by the turning of the embryo. These persist longest in the region of the heart and immediately behind it.

II. *Experimental.*

1. Destruction of the anterior ectamnionic rudiment of one side prior to the formation of the head-fold of the amnion results (*a*) in permanent absence of the amnion back to the hind-limbs (exp. 57); (*b*) in inhibition of the growth, and almost complete suppression of the folds of the amniogenous somatopleure of the uninjured side; from which we may conclude—

2. That the growth of the amniogenous somatopleure is normally induced by the traction exerted on it by the progressive fusion of the folds, and that the uprising of the folds is due to the lifting power of the same process of fusion.

3. The tail-fold and posterior lateral folds cannot replace the anterior lateral and head-folds, nor can the latter replace the former.

4. Not only the initiation, but also the progress of the formation of the anterior lateral folds is dependent upon the perfection of the head-fold (exp. 36).

5. The absence of the amnion has, at least for a time, only a limited effect on the development of the allantois.

6. Inasmuch as the embryo may develop perfectly normally to the stage of five or six days without the amnion, it is obvious that the functional significance of the latter must be slight during

this period. It yet remains to be determined how far the embryo may develop without the amnion (see quotation from Dareste below).

7. There is a certain relation of interdependence between the formation of the amnion and the body-wall. In the absence of normal formation of the lateral folds of the amnion the closure of the somatopleure to form the body-wall proceeds more slowly than usual.

Dareste ('79) has observed total absence of the amnion in embryos of the chick. The condition was not, however, produced experimentally. His observations and conclusions are given in the following quotations :

“ J'ai signalé, depuis longtemps, l'arrêt de développement de l'amnios et les anomalies nombreuses que cet arrêt partiel détermine chez l'embryon. C'est la cause la plus fréquente des monstruosités simples. Il y a des cas, beaucoup moins nombreux, il est vrai, dans lesquels l'amnios fait complètement défaut. L'embryon est alors en continuité directe, par son enveloppe cutanée, avec le feuillet séreux du blastoderme, qui ne s'est pas plissé pour former la poche amniotique. J'ai vu, dans plusieurs de ces cas, l'embryon se constituer d'une manière parfaitement normale. La paroi thoraco-abdominale s'était complètement formée, et la continuité de l'embryon avec le feuillet séreux constituait une sorte de cordon ombilical. L'allantoïde sortant de l'abdomen par se cordon s'était engagé entre le feuillet séreux et le feuillet vasculaire.”

“ Les embryons, ainsi privés d'amnios, peuvent vivre pendant un temps assez long. J'ai constaté l'absence complète de l'amnios sur un embryon de treize jours, qui était plein de vie et parfaitement normal. Rien ne pouvait faire penser qu'il mourrait prochainement. Il est très-probable cependant qu'il n'aurait pas atteint l'époque de l'éclosion. L'absence de l'amnios aurait mis obstacle au développement complet de l'allantoïde : ce qui aurait produit l'asphyxie de l'embryon, comme je l'ai montré depuis longtemps. Le plus ordinairement l'absence de l'amnios amène la mort précoce de l'embryon. Souvent aussi elle détermine, dans son organisation, des modifications tératogéniques profondes.”

“Toutes des observations nous font connaître le rôle physiologique de l'amnios dans la vie embryonnaire. Il est bien évident que l'amnios protège l'embryon contre toutes les actions mécaniques qui tendraient à le comprimer.”

HULL ZOÖLOGICAL LABORATORY, UNIVERSITY OF CHICAGO,
April, 1903.

POSTSCRIPT.

After the foregoing paper was fully printed, my attention was called to an article by Weldon in which anamniote embryos of the fowl were described, and which I had overlooked owing to the fact that the observations were included in an article entitled “Prof. de Vries on the Origin of Species” (*Biometrika*, Vol. I., Part III., April, 1902). Partial or complete suppression of the amnion resulted from experiments to replace the water lost by evaporation in the incubator without preventing the process of evaporation itself. “A hole was made in the broad end of the egg-shell and the subjacent membranes, into which one end of a siphon, filled with water, was fitted. The other end of the siphon was placed in a reservoir of water, and the whole apparatus placed in an incubator. In from 20 to 30 per cent. of the embryos treated in this way the amnion was largely or entirely absent after incubation for three or four days.”

Weldon does not discuss the mechanics of formation of the amnion, but treats the result simply as an example of a definite relation between the environment and an extremely stable character. Apparently the immediately effective factor in the experiments was the increased pressure within the shell, which, presumably, forced the embryonic area into immediate contact with the shell membrane, and thus prevented the uprising of the amniotic folds.

F. R. L.

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